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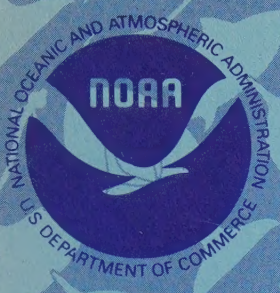

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THE AMINO ACID AGING METHOD: DOLPHIN STUDIES 1979-1980 REPORT

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The main objective of this research was to ascertain the overall reliability and precision of the amino acid racemization method for assessing the ages of dolphins. The work was divided into three categories: the comparison of the racemization ages derived from the analyses of teeth from known age mammals with their actual ages; the comparison of the rate of aspartic acid racemization in lens nucleus of various mammalian species; and, the use of the racemization method for assessing the ages of dolphins.

A series of teeth from several known age mammalian species were obtained from zoos and marine aquatic parks in the United States. The dentine was isolated and the samples were all processed using the procedure descried elsewhere for human teeth (1). Ages were calculated using the following equation (derived from the analyses of known age human teeth, see Ref. 1);

$$t(\text{yrs}) = \frac{\text{Ln}(1+D/L) - 0.014}{7.87\times10^{-4}}$$

The derived ages are listed in Table 1. (These results are also listed and discussed in Ref. 2). As can be seen, the ages deduced from racemization are in general agreement with actual ages of the various animals. It should be pointed out that racemization gives the age of the tooth, which should be slightly younger than the animal's actual age since teeth develop during the first few years of life. We feel that the agreement shown in Table 1 is acceptable, considering that the D/L ratios we are measuring are exceedingly small. The fact that the racemization derived ages are consistent with the actual ages of the various animals suggests that the rate of racemization in dentine is the same in all mammals.

These results suggest that, based on the extent of racemization in a tooth and using the k_{asp} value determined from human teeth of known age, the age of any mammal can be determined. This aging method should provide an important new tool for wildlife biologists who are often unable to determine accurately the ages of wild animals.

The next phase of our work was to compare the racemization rate in the lens nucleus of humans with that of other mammal species. The extent of racemization in both the teeth and lens nucleus of several mammalian species were analyzed. In humans, the ratio of the rate constants for racemization in tooth dentine and the lens nucleus (abbreviated Q) has a value of 0.6. Since, as summarized earlier, the rate of racemization in dentine is apparently the same in all mammals, the demonstration that Q is the same in all mammals would

thus imply that the rate of racemization in the lens nucleus is a constant regardless of the mammalian species. The Q values determined for several different mammals, including some cetaceans, are given in Table 2. As can be seen the Q values in other mammals are considerably smaller than the value for human beings. This in turn implies that the rate of racemization in the lens nucleus of other mammals is significantly faster than in humans. The reason for this is not known at present, but may be due to slight differences in the protein composition of the lens nucleus of other mammals in comparison to humans. The results in table 2 imply that the k_{asp} value in the lens nucleus of the marine mammals is on the order of 2.8 times faster than in humans, i.e., in the marine mammal lens nucleus

$$k_{asp} \quad 3.5 \times 10^{-3} yr^{-1}.$$

The amino acid method was used to estimate the ages of 16 dolphins; both teeth and lens were used to assess the ages of four animals, while only the lens were used for the remainder. The results are listed in Table 3, as are the ages estimated from growth layer counts.

The ages determined from both the teeth and lens are in good agreement. However, only two of the four ages are in reasonable agreement with the ages derived from growth layer counts. The reason for this disagreement is unknown. The fact that the racemization ages derived from the teeth and lens are concordant makes it difficult to rationalize that the racemization based ages are erroneous, since it would be highly unlikely that the contamination, or other factors, would affect both the teeth and the lens in an identical manner.

The ages estimated from only the lens analyses show a fair agreement with those derived from growth layers, if the lens which were red in coloration (probably due to hemorrhaging of the blood vessels in the retina) are discarded because of probable contamination. The ages of the Delphinus delphis samples should not be considered reliable at this point due to the lack of a suitable t = 0 sample for this species.

The ages obtained from racemization analyses provide a good check on those obtained from growth layers. As discussed above, there appears to be good agreement between the two methods for some specimens, and total disagreement for others. It is suggested that the reason(s) for this disagreement should be resolved before it can be ascertained which of the two methods provide more reliable ages. It should be emphasized that the racemization ages are estimated using equations derived from analyses of both known age human specimens and wild animals. The growth layer estimates on the other hand have utilized tetracycline fed captive animals for calibration. Whether this "calibration" is applicable to animals in their natural state is somewhat uncertain.

It is proposed that this research be continued with the aim of resolving the disagreement between the ages derived from racemization and growth layers. In particular it is suggested that the teeth and lens from the same animal be used to calculate an average racemization age. These ages could then be compared with those determined from growth layers, and hopefully these further analyses will help establish which of the two dating methods give the more

precise age estimates.

REFERENCES

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J. L. Bada and S. L. Brown, Trends in Biochem. Sci., in press.

Table 1. Age estimates of various mammalian species of known age as determined from the extent of aspartic acid racemization in dentine.

Species	D/L Aspartic acid	Aspartic acid age(yrs)*	Actual age (yrs)+
Callorhinus ursinus (northern fur seal)	0.0361	27	14+
Macaca nemestrina (monkey)	0.349	26	20+
Macaca nemestrina (monkey)	0.0231	11	4
Macaca nemestrina (monkey)	0.020	7	10
Camelus dromedarius (camel)	0.0361	27	20
Eguus burchelli bohmi (zebra)	0.0335	24	27
Gorilla gorilla (gorilla)	0.0341	25	30
Zalophus californianus (California sea lion)	0.0375	29	25+
Stenella attenuata (spotted dolphin)	0.014	0	0

*Calculated from $t(yrs) = \frac{Ln(1+D/L) - 0.014}{7.87 \times 10^{-4}\ yr^{-1}}$

+Obtained from zoo records

Table 2. Species differences in the ratio of aspartic acid racemization rates in dentine and lens proteins.

Species	$k_{dentine}/k_{lens\ nucleus}=Q$
Homo sapiens	0.63
Equus burchelli bohmi (zebra)	0.31
Stenella attenuata (spotted dolphin)	0.20
Callorhinus ursinus (northern fur seal)	0.23
Delphinapterus leucas (white whale)	0.24

Table 3. Age estimates for dolphins derived from racemization analyses of teeth* and lens.+

Specimen #	D/L aspartic acid	Aspartic age (yrs)	Age from growth layers (yrs)++
		Stenella attenuata	
DLV-0012-lens	0.0335	1	
DLV-0012-tooth	0.0161	3	9
DLV-0015-lens	0.0281	0	
DLV-0015-tooth	0.0132	0	2.5
DLV-0018-lens	0.0574	7	
DLV-0018-tooth	0.020	7	18-19
DLV-0037-lens	0.103	19	
DLV-0037-tooth	0.0274	17	19
WAW-560-lens	0.0308	0	?
RMB-024-lens**	0.0778	13	16
WAW-569-lens	0.0304	used for t=0	0.8
RMB-047-lens**	0.0571	7	21-23
ASO-011-lens	0.0754	12	18
JJN-004-lens***	0.0605	8	21-23
		Stenella longirostris	
WAW-586-lens	0.0498	6	7
WAW-585-lens	0.0791	14	11-12?
WAW-587-lens	0.0587	9	6-7
JRE-016-lens	0.275???	?	?
		Delphinus delphis	
JRE-017-lens	0.117	23	10?
JRE-018-lens	0.0849	15	5?

*The ages of the teeth were calculated from the equation

$$t(\text{yrs}) = \frac{\text{Ln}\,(1+D/L) - 0.014}{7.97\times10^{-4}}$$

+The lens ages were calculated from the equation

$$t(\text{yrs}) = \frac{\text{Ln}\,(1+D/L) - \text{Ln}\,(1+D/L)_{t=0}}{3.5\times10^{-3}}$$

where specimen #WAW-569 was used to calculate the t=0 term for *S. attenuata* and a D/L=0.026 was used to calculate the t-0 term *S. longirostris*. There is no t=0 sample for *D. delphis*, so the value for *S. longirostris* was used for this species.

**Lens was red, although the core was white

***Lens was red, including core

++Provided by A. Myrick Jr., SWFC